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ADDITIONS TO FUNGI OF MADRAS—XII

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Received March 16, 1951

Endodothella kanarensis Ramakrishnan and Sundaram sp. nov.

Stromata black, epiphyllous, 1–2 mm. across, isolated or clustered, irregular, raised, clypeate, shining, pluriloculate, occupying three-fourths of the thickness of the leaflet; locules globose or flattened, 110–250 μ high and 110–270 μ broad, ostiolate; asci clavate, thin walled, wall gelatinising, 82 × 14 μ (60–105 × 9–18), paraphysate, paraphyses filiform; ascospores irregularly biseriate, spindle-shaped, 2-celled, hyaline, constricted at the septum, 16 × 6 μ (12–21 × 6–9).

Stromata nigra, epiphylla, 1–2 mm. lata, dispersa vel aggregata, irregularia, elevata, clypeata, micantia, pluriloculata, tres quartas partes crassitudinis foliolorum occupantia; loculi globosi vel applanati, 110–250 μ alti atque 110–270 μ lati, ostiolati; asci clavati, tenuiter parietati, parietibus gelatinosis, 82 × 14 μ (60–105 × 9–18), paraphysati, paraphysibus filiformibus; ascosporæ irregulariter biseriatæ, fusiformes, 2-cellulatæ, hyalinæ, constrictæ ad septa, 16 × 6 μ (12–21 × 6–9).

On living leaflets of *Albizzia odoratissima* Benth. (Mimoscideæ), S. Kanara, 2-2-1951, N. V. Sundaram.

The stromata occur on the upper surface of the leaflets as raised black shining formations. They are of varying shapes and sometimes neighbouring stromata fuse together. One to five loculi have been observed in each stroma. The ascus wall gelatinises early and the ascospores are found loose in the loculi. The spores taper towards the two ends and are surrounded by mucilage. Theissen and Sydow (1915) have described *E. albizziæ* on *Albizzia marginata* from the Philippines. But the fungus differs from it in the structure of the stroma and the size and other characters of the asci and ascospores.

Phyllachora hugoniæ Theiss. and Syd.

Theissen, F. and Sydow, H., *Ann. Myc.*, 1915, **13**, 512.

On leaves of *Hugonia mystax* L. (Linaceæ), Tirupathi, 8-2-1951, T. S. Ramakrishnan.

Physalospora clerodendri sp. nov.

Spots foliicolous, small upto 2 mm. in diam. yellowish, thickened, with one perithecium in the middle of each spot; perithecium black ostiolate, deep seated upto 530 μ wide and 320 μ high; asci cylindric clavate, hyaline, 8 spored, 50 × 17 μ (44–60 × 12–20), gelatinising, paraphysate, paraphyses filiform; ascospores elliptic to oblong, hyaline, one-celled, uniseriate, 9 × 7 μ (5–14 × 5–8).

Maculæ foliicolæ, parvæ, usque ad 2 mm. diam., luteolæ, crassiusculæ, uno perithecio in medio uniuscuiusque maculæ; perithecium nigrum, ostiolatum, alte insitum, usque ad 530 μ latum et 320 μ altum; asci cylindrici, clavati, hyalini, octospori, 50 × 17 μ (44–60 × 12–20), gelatinosi, paraphysati, paraphysibus filiformibus; ascosporæ ellipticæ vel oblongæ, hyalinæ, 1-cellulatæ, uniseriatæ, 9 × 7 μ (5–14 × 5–8).

On living leaves of *Clerodendron infortunatum* L. (Verbenaceæ), Mundaje (S. Kanara), 12–12–1950, T. S. Ramakrishnan.

The spots are thickened and circular with one perithecium embedded in the centre. At the top and the base of the perithecium black stromatic tissue is evident but the sides are not coloured. The perithecium occupies almost the entire thickness of the leaf. Crowding on the sides and near the ostiole are numerous filamentous paraphyses with orange coloured contents. The asci gelatinise early and the ascospores are seen in groups in the loculus or escaping through the ostiole embedded in mucilage. The ascus can be seen clearly only in the young perithecia.

Ustilaginoidea oplismeni Ramakrishnan and Sundaram sp. nov.

Ovaricolous, elliptical, greenish black, upto 2 mm. long and 0·75 mm. broad, centre white to orange brown; conidia globose, olive brown, verrucose, 6 μ (5–7) in diam.

Ovariicola, elliptica, virido-nigra, usque ad 2 mm. longa, 0·75 mm. lata, centro albo vel brunneo-citreo; conidia globosa, olivaceo-brunnea, verrucosa, 6 μ (5–7) diam.

Infecting ovaries of *Oplismenus burmannii* Beauv. (Gramineæ), Walayar (Malabar), 16–12–1950, N. V. Sundaram.

Complete infection of all spikelets of the panicle is common. The spores germinate readily producing germ tubes which branch freely and give rise to clusters of hyaline, one-celled, elliptic conidia.

Sorosporium brachiariæ-ramosæ sp. nov.

Sori ovaricolous, infecting all the spikelets, covered by a false membrane, columella 3-4 branched from the base, spores in semi-permanent globose masses, 42 × 31 μ (26–63 × 17–50) in diam. having many spores in each mass; spores globose or angular, 12 × 11 μ (10–15 × 9–13) diam., reddish brown, epispore finely verrucose.

Sori ovariicoli, spicules omnes efficientes, operte membrana falsa quæ nempitur, prædiți columella tribus vel quattuor ramis ornata quæ surgit ex basi; sporæ in globulis semipermanentibus, 42 × 31 μ (26–63 × 17–50) sporarum numero constantibus; sporæ globosæ ad angularos, 12 × 11 μ (10–15 × 9–13) diam., rufescentes brunneæ, episporio subtiliter verrucoso.

In ovaries of *Brachiaria ramosa* Stapf (Gramineæ), Pollachi, 20-8-1912, S. Sundararaman.

This smut affects all the spikelets in the panicle. The sori protrude as dirty grey sacs from between the lemma and the palea. The false membrane covering the sorus splits irregularly from the tip exposing the dark brown mass of spores. The spores are agglutinated into semi-permanent masses. In immature sori the spore masses are surrounded by layers of thin hyphæ. Many of the spore masses have persisted as such for over 38 years in the herbarium material. Projecting from the base of the sorus are three to four columellar branches united at the base. The characters of the smut indicate that it belongs to the genus *Sorosporium*. This differs from other smuts on *Brachiaria*.

Sphacelotheca erythraciens (Syd.), Clint. in *N. Am. Flora.*, 1939, **7**, 996.

On the inflorescences of *Hackelochloa granularis* O. Ktz. (*Manisuris granularis* L.) (Gramineæ), Chittoor, 12-11-1950, G. S. Reddy.

All the ears of a plant are infected. The spikes are converted into long dark brown sori enclosed in sheaths. A central columella is present; this is usually simple but sometimes short lateral branches are present at the upper half.

Sphacelotheca fagopyri Syd. and Butl.

Sydow, H. and P., and Butler, E. J., *Ann. Myc.*, 1907, **5**, 486.

In ovaries of *Fagopyrum esculentum* Moench. (Polygonaceæ), Keti (Nilgiris), 2-10-1950, N. V. Sundaram.

The sorus wall splits at the apex and becomes reflexed exposing the violet black mass of spores. There is a stout central columella. All the

ovaries are infected and mycelium is present in the tissue of the stem also, indicating systemic infection.

Ustilago operta Syd. and Butler

Sydow H. and P., and Butler, E. J., *Ann. Myc.*, 1906, **4**, 426.

In ovaries of *Brachiaria ramosa* Stapf (Gramineæ), Chittoor, 11-11-1950, G. S. Reddy.

The infection is confined to some of the spikelets in the inflorescence. The sori are greenish, smooth and provided with a short blunt, columella. The sorus wall is composed of 3–4 layers of host cells with an internal lining of fungal tissues.

Cerotelium fici (Cast.) Arth.

Butler, E. J. As *Kuchneola fici* (Cast.), Butl. in *Ann. Myc.*, 1914, **12**, 76.

On leaves of *Morus indica* L. (Moraceæ), Kallar Fruit Gardens, 1-1-1951, N. V. Sundaram.

Small brown spots are formed on the leaves. On the lower surface of these are numerous minute conical uredia, isolated or in groups. White spore masses can be seen collected on the burst sori. Uredia alone were present and these resembled the uredia of *C. fici*.

Kuehneola trichosanthes (Petch) Ramakrishnan and Sundaram comb. nov.

Pycnia and æcia not seen. Uredia hypophyllous, minute, 0·1–0·2 mm. in diam., subepidermal, erumpent, orange coloured; urediospores obovate to globose, short stalked, sparsely echinulate, wall hyaline and contents orange coloured, 25–34 × 22–28 μ, germpores 2, paraphysate, paraphyses peripheral, cylindric, subhyaline; telia hypophyllous, minute, whitish, subepidermal, erumpent, aparaphysate; teliospores catenulate, forming chains of 4–5, almost hyaline, each cell oblong with a projection on one side at the top, 25–43 × 11–15 μ, germinating *in situ*; basidium 4-celled, basidiospores elliptic to globose.

Pycnia atque æcia ignota. Uredia hypophylla, minuta, 0·1–0·2 mm. diam., subepidermalia, erumpentia, aurantiaca; uredosporæ obovatæ vel globosæ, breviter pediculatæ, sparse echinulatæ, parietibus hyalinis atque contentis aurantiacis, 25–34 × 22–28 μ, germinationis poris duobus ornatæ, paraphysatæ, paraphysibus peripheralibus, cylindricis, subphyalinis; telia hypophylla, minuta, albida, subepidermalia, erumpentia, aparaphysata; teliosporæ catenulatæ, catenas 4–5 cellularum efformantes, prope hyalinæ, singulis cellulis oblongis, atque in apice ornatis lateraliter projectione

quadam, 25–43 × 11–15 μ; teliosporæ germinant *in situ*, basidiis 4-cellulatis, basidiosporæ ellipticæ vel globosæ.

On leaves of *Trichosanthes palmata* Roxb. (Cucurbitaceæ), Kallar (Coimbatore), 1-1-1951, N. V. Sundaram.

Numerous sori develop on the lower surface of the leaves. Uredia and telia are mixed together but can be easily distinguished by the difference in colour. Urediospores are produced singly on short stalks. On the margins of the sori thin-walled paraphyses can be seen. The teliospores germinate *in situ* the topmost cells germinating first. The germinating spores contribute to the colour of the telia. The chains are not laterally united.

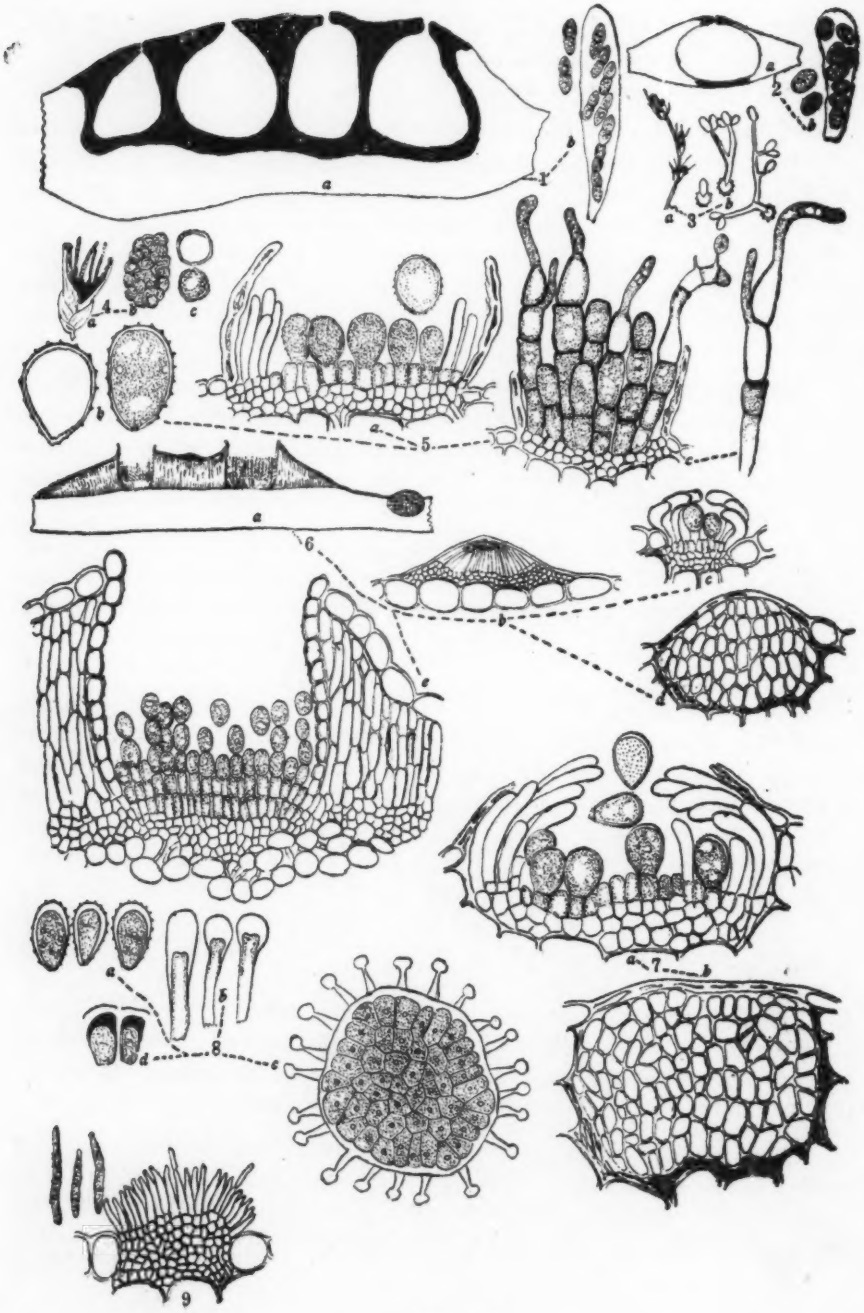
Though this genus was originally described as being confined to Rosaceous hosts its occurrence on Malvaceæ and Anacardiaceæ have been recorded from Africa and Ceylon respectively. The present rust is on another host belonging to *Cucurbitaceæ*. Thus the genus has a wider host range than originally contemplated.

Petch (1912) has described *Uredo trichosanthes* on this host from Ceylon. It has not been possible to obtain type specimens of this material for comparison but its description is in agreement with the uredial stage of the rust under study. Therefore a new combination has been effected.

Phakopsora chorisandræ Ramakrishnan and Reddy sp. nov.

Pycnia minute, dark, subcuticular, amphigenous, hemispherical or conical, 100 μ broad and 33 μ high (68–200 × 20–48); aecia hypophyllous in clusters of 6–10, whitish, cupulate, subepidermal, 205 × 200 μ (160–254 × 144–254), peridiate, peridium of one layer of cells which are polygonal, prominently verrucose, hyaline, 17 × 12 μ (12–24 × 8–14); æciospores catenulate, elliptical or oblong, thin and smooth-walled, subhyaline, 15 × 14 μ (10–19 × 8–17); uredia few, hypophyllous, white, pulverulent, subepidermal, erumpent, paraphysate, paraphyses incurved, clavate, subhyaline; urediospore subhyaline, subglobose, subsessile, thin-walled, finely echinulate, 10–12 μ diam.; telia numerous, hypophyllous, minute, lenticular, dark, subepidermal, 60–100 μ wide and 62–100 μ high; teliospores sessile, many layered, not catenulate, polygonal, reddish brown in colour, 16 × 11 μ (11–20 × 9–13), smooth-walled.

Pycnia minuta, fusca, subcuticularia, amphigena, hemisphærica vel conica, 100 μ lata, 33 μ alta (68–200 × 20–48); æcia hypophylla, 6–10 aggregata, alba, cupulata, subepidermalia, 205 × 200 μ (160–254 × 144–254), peridiata, peridio constanti unica serie cellularum polygonaliam, fortiter



FIGS. 1–9

FIGS. 1–9. Fig. 1. *Endodothella kanarensis*, *a*. section of stroma (diagrammatic), *b*. ascus and ascospores (× 300). Fig. 2. *Physalospora clerodendri*, *a*. section of perithecium (diagrammatic), *b*. ascus and ascospores (× 300). Fig. 3. *Ustilaginoidea oplismeni*, *a*. infected spikelets, *b*. spores germinating (× 300). Fig. 4. *Sorosporium brachiariæ-ramosæ*, *a*. infected spikelet, *b*. spore ball (× 200), *c*. spore (× 500). Fig. 5. *Kuehneola trichosanthes*, *a*. section of uredium (× 300), *b*. urediospores (× 600), *c*. section through telium and one chain of teliospores (× 300). Fig. 6. *Phakopsora chorisandræ*, *a*. section showing æcia, telium and pycnium (diagrammatic), *b*. section through *b*. pycnium, *c*. uredium, *d*. telium and *e*. æcium (× 300). Fig. 7. *Phakopsora mangalorica*, section through *a*. uredium and *b*. telium (× 300). Fig. 8. *Ravenelia coimbatorica*, *a*. urediospores, *b*. paraphyses, *c*. telial head and *d*. teliospores (× 300). Fig. 9. *Cercospora chloroxyli*, stroma and conidia (× 300).

verrucosarum, hyalinarum, 17 × 12 μ (12–24 × 8–14); æciosporæ catenulatæ, ellipticæ ad oblongæ, tenui atque levi pariete ornatæ, subhyalinæ, 15 × 14 μ (10–19 × 8–17); uredia pauciora, hypophylla, albida, pulverulenta, subepidermalia, erumpentia, paraphysata, paraphysibus incurvis, clavatis, subhyalinis; uredosporæ subhyalinæ, subglobosæ, subsessiles, tenuiter parietatæ, leviter echinulatæ, 10–12 μ diam.; telia plurima, hypophylla, minuta, lenticularia, fusca, subepidermalia, 60–100 μ lata, 62–100 μ alta; teliosporæ sessiles, multi-seriatæ, haud catenulatæ, polygonales, rubro-brunneæ colore, 16 × 11 μ (11–20 × 9–13), parietibus levibus præditæ.

On living leaves of *Chorisandra pinnata* W., Chittoor, 12-11-1950, G. S. Reddy.

Heavy infection of the leaves of the host, *Chorisandra pinnata* had occurred. Yellowing of leaves and defoliation were evident. This rust produced all the types of sori and spores and was thus of particular interest. The sori developed near each other on the same leaf so that there was no room to doubt their relationship.

Pycnia are amphigenous appearing as dark dots scattered over the surface. They are subcuticular, hemispherical or slightly conical with reddish brown contents. Ostiolar filaments are not evident. Aecia develop in clusters hypophyllously. Circular indefinite yellowish spots on the upper surface denote the location of these sori. These are cup-shaped, white and occur in groups of 6–10 or more. The tissue of the leaf is thickened owing to the development of vertical plates of hyphæ between the epidermis and the mesophyll, all round the æcia. The æcia are typical developing subepidermally with a well-defined peridium of one layer of hyaline, polygonal, prominently verrucose cells.

Uredia are rarer but can be detected in young infections as minute, pulverulent whitish circular sori on the lower surface. The sori are subepidermal, erumpent and surrounded by several series of incurved clavate

subhyaline paraphyses enclosing a few subhyaline or hyaline elliptic urediospores.

Telia are formed in large numbers hypophyllously. They occur in groups as dark violet lenticular crusts. The teliospores are in many layers but not catenulate, one-celled, polygonal and reddish brown in colour.

The nature of the uredia and telia enables the identification of this rust as a species of *Phakopsora*. Pycnia and æcia have not been seen in the species at present included in this genus.

The present rust has both these types of sori and the æcia are typical and different from the uredinoid æcia reported for *Bubakia*. The differences in the structure of the æcia and uredia are sufficiently significant to keep the two genera distinct from each other.

Phakopsora desmium (B. and Br.) Cummins.

Cummins, G. B., *Bull. Torr. Bot. Club*, 1945, **42**, 406.

On leaves of *Gossypium arboreum* L. (Malvaceæ). S. Kanara, 9–12–1950, T. S. Ramakrishnan; on leaves of *G. arboreum* L. (Karunganni), Tirunelveli, 9–2–1951, K. Sundaram.

Phakopsora mangalorica Ramakrishnan and Sundaram sp. nov.

Pycnia and æcia not found. Uredia mainly hypophyllous scattered in discoloured areas, minute, 0·1 to 0·4 mm. in diam., brown, conical, subepidermal, later erumpent, bound by numerous incurved clavate paraphyses, paraphyses subhyaline, sometimes wall thickened at the upper portion. 28 × 8 μ (19–40 × 6–12); urediospores subglobose, obovate or elliptic, light brown, wall coloured, finely echinulate, short stalked, 22 × 17 μ (19–28 × 16–22), pores indistinct; telia amphigenous, scattered or clustered, 0·05 to 0·3 mm. in diam., lenticular, dark brown, subepidermal, nonerumpent; teliospores one-celled, compactly arranged in five to seven layers, not catenulate, sessile, polygonal, vinaceous brown, wall coloured, 18 × 12 μ (12–22 × 9–16).

Pycnia atque æcia ignota. Uredia ut plurimum hypophylla, dispersa per areas discoloratas, minuta, 0·1–0·4 mm. diam., brunnea, conica, subepidermalia, tandem erumpentia, circumscripta plurimis incurvis clavatis paraphysibus subhyalinis, nonnumquam parietibus incrassatis in superiore parte, 28 × 8 μ (19–40 × 6–12); uredosporæ subglobosæ, obovatæ vel ellipticæ, tenuiter brunneæ, parietibus coloratis, tenuiter echinulatis, breviter pedicellatæ, 22 × 17 μ (19–28 × 16–22), poris indistinctis; telia amphigena, dispersa vel aggregata, 0·05–0·3 mm. diam., lenticularia, fusce brunnea,

subepidermalia, non-erumpentia; teliosporæ 1-cellulatæ, compacte dispositæ in 5–7 tabulata, haud-catenulatæ, sessiles, polygonales, vinaceo-brunneæ, parietibus coloratis, 18 × 12 μ (12–22 × 9–16).

On leaves of *Desmodium triquetrum* DC. (Papilionatæ), S. Kanara, 2-2-1951, N. V. Sundaram.

This rust is common in S. Kanara. The telia are prominent on both sides of the leaves. *P. meibomiæ* Arth. has been recorded on other species of *Desmodium* from America and the Philippines. The rust under study differs from this in many respects for example in the position of the sori, number of layers of telial cells, colour and size of urediospores, etc.

Puccinia malvacearum Mont.

Sydow, P. and H., *Monogr. Ured.*, 1904, **1**, 476.

On leaves of *Malva verticillata* L. (Malvaceæ), Keti, 2-10-1950, N. V. Sundaram.

Telia were present and most of the spores had germinated.

Puccinia nepalensis Barcl. and Diet.

Sydow, P. and H., *Monogr. Ured.*, 1904, **1**, 578.

On leaves and petioles of *Rumex nepalensis* Spr. (Polygonaceæ), Ootacamund, 16-1-1951, T. S. Ramakrishnan.

Uredia alone were present. These were formed in large numbers, being located hypophyllously in the middle of red spots. Uredia were seen on the petioles also.

Ravenelia coimbatorica Ramakrishnan and Sundaram sp. nov.

Pycnia and æcia not found. Uredia amphigenous, minute, circular, clustered, subepidermal, erumpent; urediospores oblong, fusiform, broader in the upper half, apex often thickened, light brown, pedicellate, 25 × 15 μ (22–40 × 12–19), finely echinulate, with 6–8 scattered germ pores; paraphyses innumerable, surrounding and mixed with the urediospores, clavate to spatulate, the bulbous portion brown and thickened up to 16 μ, 76 × 14 μ (62–93 × 9–19); telia amphigenous, near uredia, dark, subepidermal, erumpent, aparaphysate, teliospore heads chestnut brown 74–118 μ, up to 10 cells across in the head, compound pedicel, 14 × 6 μ (9–12 × 3–6), marginal hyaline papillæ swollen at the apices; spores one-celled 30 × 18 μ (22–37 × 12–25), wall coloured, thickened up to 10 μ at the top, cysts hyaline, round and pendulous.

Pycnia atque æcia ignota. Uredia amphigena, minuta, circularia, aggregata, subepidermalia, erumpentia; uredosporæ oblongæ vel fusiformes, latiores in superiore parte, apice sæpe incrassato, pallide brunneæ, pedicellatæ, 25–15 μ (22–40 × 12–19), tenuiter echinulatæ, germinationis poris 6–8 dispersis ornatæ; paraphyses innumeræ uredosporas circumdantes vel cum eisdem mixtæ, clavatæ vel spathulatæ, parte bulbosa brunnea atque incrassata ad 16 μ, 76 × 14 μ (62–93 × 9–19); telia amphigena, prope uredia, obscura, subepidermalia, erumpentia, aparaphysata; teliosporarum capitula castaneo-brunnea, 74–118 μ, 10 cellulis in diametro capituli positis; pediculus compositus, 14 × 6 μ (9–12 × 3–6), ornatus marginalibus papillis hyalinis incrassatis in apice; sporæ 1-cellulatæ, 30 × 18 μ (22–37 × 12–25), parietibus coloratis, incrassatis usque ad 10 μ in apice, cystis hyalinis, rotundis atque pendulis.

On leaves of *Phyllanthus urinaria* L. (Euphorbiaceæ), Kallar, 1-1-1951, Sri. N. V. Sundaram.

Uredia occur in groups and are reddish brown in colour on small dark brown spots. Each uredium is almost round with a raised ring of densely arranged paraphyses along the margin and depressed centre. The urediospores are stalked and mixed with the paraphyses. Telia are formed close by the uredia but appear darker. The telium is surrounded by a ring of ruptured epidermis. This rust differs from *R. phyllanthi* Mund. and Thirum. (Mundkur and Thirumalachar, 1946) in having fusiform and bigger urediospores.

Uredo artocarpi B. and Br.

Petch, T., *Ann. Roy. Bot. Gard., Peradeniya*, 1912, **5**, 252.

On leaves of *Artocarpus integrifolia* L. (Moraceæ), Adathorai estate, Nilgiris, 16-1-1951, T. S. Ramakrishnan.

Numerous minute brown irregular spots are formed on the leaves. These are visible on both sides of the leaves. Uredia are formed as minute raised sori hypophyllously, one to three in a spot.

Uredo elephantopodis Petch.

Petch, T., *Ann. Roy. Bot. Gard., Peradeniya*, 1912, **5**, 249.

On leaves of *Elephantopus scaber* L., Mangalore, 12-12-1950, T. S. Ramakrishnan.

The uredia have incurved uniseptate marginal paraphyses.

Uromyces andropogonis-annulati Syd. and Butl.

Sydow, H. and P. and Butler, E. J., *Ann. Myc.*, 1907, 5, 492.

On leaves of *Andropogon longipes* Hack. (Gramineæ), Ootacamund, 15-2-1950, T. S. Ramakrishnan.

This rust was common on this grass in many places on the Nilgiris. Uredia alone were present. Dr. Cummins of Purdue University to whom specimens were sent places the rust in this species.

Cercospora chloroxyli Ramakrishnan and Reddy sp. nov.

Spots irregular, amphigenous, light brown; clusters of conidiophores hypophyllous, originating from a subepidermal stroma, dark; conidiophores cylindric, usually nonseptate, sometimes with one septum, olivaceous brown, 21 × 5 μ (12–33 × 5–7), numerous in each cluster; conidia cylindric, rounded at apex, 1–4 septate, subhyaline to light brown, 45 × 5 μ (31–62 × 3–7).

Maculæ irregulares, amphigenæ, pallide brunneæ; conidiophororum acervi hypophylli, ex subepidermali stromate obscuro originates; conidiophori cylindrici, ut plurimum non-septati, nonnumquam uno septo ornati, olivaceo-brunnei, 21 × 5 μ (12–33 × 5–7), plures in singulis acervis; conidia cylindrica, rotundata in apice, 1–4-septata, subhyalina vel pallide brunnea, 45 × 5 μ (31–62 × 3–7).

On leaves of *Chloroxylon swietenia* DC. (Rutaceæ), Kallar, 1-1-1951, G. S. Reddy.

The spots are straw coloured and studded with numerous dark conidiophore clusters on the lower surface. These are usually arranged in concentric rings.

I am indebted to Dr. G. R. Bisby for valuable suggestions and to Rev. Fr. H. Santapau for the Latin translations.

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STUDIES IN THE PHYSIOLOGY OF PHANEROGAMIC PARASITISM WITH SPECIAL REFERENCE TO *STRIGA LUTEA*, LOUR. AND *S. DENSIFLORA*, BENTH. ON *ANDROPOGON SORGHUM*, HACK.

1. The Osmotic Pressure of the Host and Parasite in Relation to the Nutrition of the Host

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[Communicated by Prof. L. S. S. Kumar, M.Sc. (Lond.), F.A.Sc.]

INTRODUCTION

THE phanerogamic parasite, *Striga*, is known to occur throughout the tropical and sub-tropical regions of Africa, Asia and Australia. Several species exist and some of them have been reported to cause considerable damage to cultivated crops. Four species of *Striga* are described in Indian floras, *viz.*, *S. lutea*, Lour., *S. densiflora*, Benth., *S. euphrasioides*, Benth., and *S. orobanchoides*, Benth. Of these, the first three have been reported to occur on economic crops like *Sorghum*, maize, sugarcane and other millets. Subsequent observations, however, have shown that only *S. lutea* and *S. densiflora* do considerable harm to these crops. The occurrence of *S. euphrasioides* in a virulent form is rare.

In India, the Great Millet (*Andropogon Sorghum*, Hack.) forms the principal cultivated host of *Striga*. Reports of the depredations of this parasite have recently increased in Bombay State. The Economic Botanist to Government, Bombay State, took up the work of evolving strains of *Sorghum* resistant to *Striga* attack in 1933. As the work progressed, it became apparent that a thorough study of the factors affecting the germination, growth and attack of *Striga* species was a necessary preliminary to carrying out a breeding programme. A special scheme was, therefore, launched for this purpose. The investigations presented in this series were carried out in the author's capacity as Investigator in the Scheme for Investigation into the attack on *Andropogon sorghum* by the parasite *Striga* financed jointly by the Indian Council of Agricultural Research and the Government of Bombay. The following is the first article in the series and will be followed by others.

The Osmotic Pressure of the Host and Parasite in Relation to the Nutrition of the Host

According to Macdougal and Cannon (1910), a higher osmotic pressure on the part of a potential parasite is a pre-condition to its entrance into nutritive couple with another plant. In order to ensure continuity of parasitism for a considerable length of time, the parasite must have the power of adjusting the strength of its own sap to any variations which may occur in that of the host. Harris and Lawrence (1916) studied the osmotic relationships of seven species belonging to three genera of the *Loranthaceæ* parasitic on 19 species of hosts and found that " the osmotic pressure of the sap extracted from the chlorophyll bearing tissues of the parasite is almost always, but apparently not invariably, greater than that expressed from the mature leaves of the host ". They conclude that " while higher osmotic pressure of the sap of the parasite is a general condition, it is not a *necessary* condition for at least the temporary success of the parasite ". Harris, *et al.* (1921) found that the osmotic strength of the tissue fluid of *Cuscuta* growing on *Impatiens* in saturated soil, is higher than that of the host; while *Cuscuta salina* growing on a halophyte, *Alleurolfea occidentalis*, has a lower sap strength than the host. Parija and Saran (1933) have investigated the extent to which the osmotic pressure of the sap of *Cuscuta reflexa* Roxb. could vary when twigs of the host were supplied with various concentrations of grape-sugar to raise the osmotic pressure of the sap of the host. They found that the sap of the parasite rose in strength but to a limit. If the strength of the host sap was raised further, the parasite sap showed no further rise but the quantity of starch in the cells of the parasite increased. They, however, conclude that " 24 hours may not be long enough for the parasite to adapt itself to the changed osmotic conditions of the host. In nature, the parasite could perhaps be educated to adapt itself to hosts having higher osmotic conditions ".

While studying the influence of nutrition of the host on the susceptibility of *Andropogon sorghum* to attack by *Striga lutea* and *S. densiflora*, the hosts along with the parasites were grown in sand cultures watered by various concentrations of the same nutrient solution for a period of nearly five months, *i.e.*, from sowing to harvest. These cultures afforded an excellent opportunity of testing the ability of the parasite to adjust its osmotic pressure to that of the host.

Material and Methods

Seeds of a susceptible variety of the host (*Maldandi* 35–1) were germinated in the laboratory and on the fifth day, seedlings of uniform size were transplanted to earthenware pots filled with pure quartz sand of

100 mesh. The sand in the pots was alternatively infected with 0·1 gram seed of *S. lutea* or *S. densiflora* or kept free. The pots were arranged on the glass-house bench according to Fisher's randomised method and watered with tap water for four days. Thereafter, the pots were watered with nutrient solutions made up as follows:—

		c.c. of M/2 stock solution required to produce 1,000 c.c. of nutrient solution
Potassium biphosphate	—KH_2PO_4 ..	26
Calcium nitrate	—$Ca(NO_3)_2$ ..	22
Magnesium sulphate	—$MgSO_4$ ..	8
Ammonium sulphate	—$(NH_4)_2SO_4$ ..	7

The pH of the solution was 5·6. The above stock solution (designated double nutrient or 2N) was diluted to half, one-quarter and one-eighth and designated N, $\frac{1}{2}$N and $\frac{1}{4}$N respectively. There were six pots for each treatment which varied in two ways, *viz.*, concentration of the nutrient solution and infection with *S. lutea* or *S. densiflora* or absence of any infection. The temperature of the glass-house ranged from 25° to 35° C. during the experiment. Each pot got three waterings a week with one of these solutions. To keep the sand moist tap water was added whenever necessary. *Striga* plants started appearing above the surface about one month after starting the experiment. Thereafter, periodical observations were made on the total number and height of *Striga* plants in each pot. After the harvest, the number of grains produced by each host plant was recorded as also the weight of the straw.

As it was not possible to use the roots of the host and the haustorial tissue of the parasite without destroying the material (which was required for determining the yield of the host and measuring the heights attained by the parasites), the osmotic pressures of the leaves of the host and the parasite were determined instead. The osmotic pressure determinations were made when the hosts had eared and when the parasites were in flower. Of the host, the third leaf from the top was used and of the parasite the topmost leaf. As far as possible leaves of nearly equal height (measured from the surface of the pot) were selected. The osmotic pressures of the host and parasite were measured simultaneously.

The plasmolytic method of DeVries (1844) as modified by Hofler (1918) was adopted. The osmotic strength of the tissue was measured by determining the molecular concentration of a cane sugar solution required to produce incipient plasmolysis. This value was converted into its equivalen

in atmospheres by using the table given by Molz (1926). Working from 8 A.M. to 6 P.M., about eight values could be conveniently determined in a day, *i.e.*, it took about 15 days to complete all the determinations. The order for determining the osmotic concentrations of the four nutrient levels for six replications was randomised, but all the values for each level, *i.e.*, free host, host attacked by *S. lutea*, host attacked by *S. densiflora*, *S. lutea* and *S. densiflora* were determined in as close an interval of time as possible. Strips were cut parallel to the central vein of the leaves by means of a sharp razor blade; these were then dipped in a very dilute solution of neutral red in order to colour the cell-sap and to facilitate detection of incipient plasmolysis. The strips were then removed for half-an-hour to watch-glasses containing various concentrations of cane sugar before examination under the microscope.

Presentation of Results

The mean values of the number of *Striga* plants appearing in the different treatments have been presented in Fig. 1. As the values obtained in this experiment are small whole numbers the square roots of these values

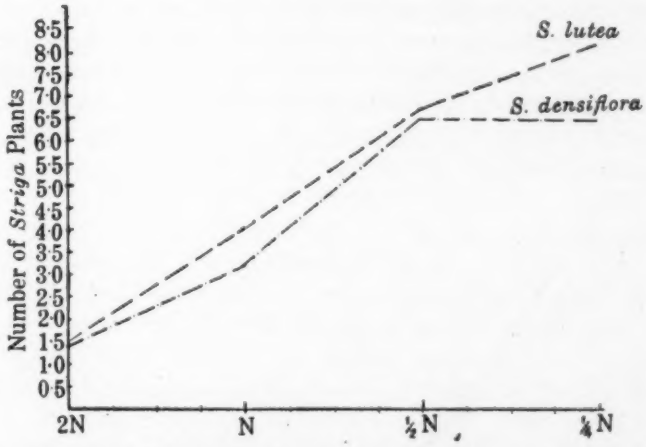

Fig. 1. The effect of four different nutrient concentrations on the number of *Striga lutea* and *S. densiflora* plants attacking a single host plant.

were employed for calculating the analysis of variance as recommended by Cochran (1938). This analysis revealed that the number of *Striga* plants attacking a single host plant is significantly larger at the lower concentrations of ¼N and ½N than at N and 2N. The least number appears at 2N. Both *S. lutea* and *S. densiflora* behave similarly in this respect.

Fig. 2 shows the total height attained by all *Striga* plants attacking a single host plant in the different treatments. An analysis of this data

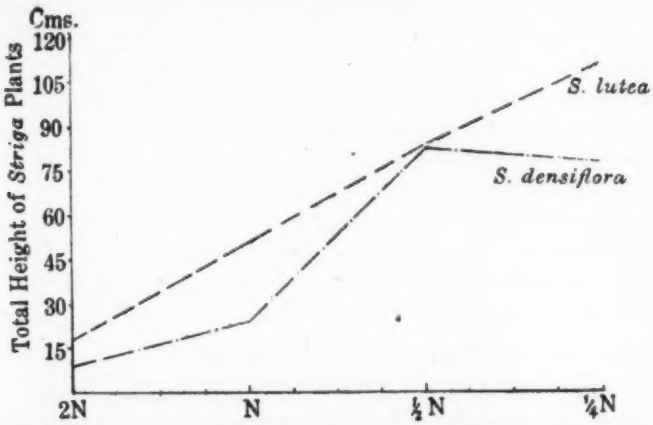

FIG. 2. The effect of four different nutrient concentrations on the total height of all *Striga lutea* and *S. densiflora* plants attacking a single host plant.

showed that the total height attained by all *Striga* plants attacking a single host plant, is significantly greater in the lower concentrations of ¼N and ½N than in the higher concentrations of N and 2N. There is no significant difference in the behaviour of *S. lutea* and *S. densiflora* in this respect as well.

The yield of grain and straw of *Sorghum* plants for the different treatments have been summarized in Figs. 3 and 4. The relevant data were

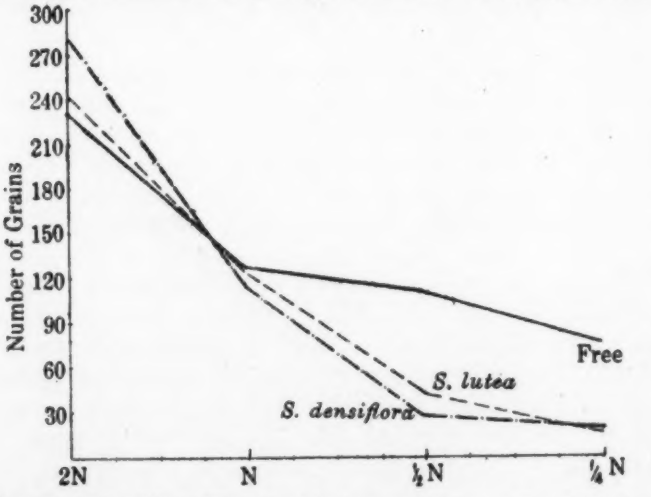

FIG. 3. The effect of four different nutrient concentrations on the yield of grain of host plants attacked by *Striga lutea* and *S. densiflora* and free host plants.

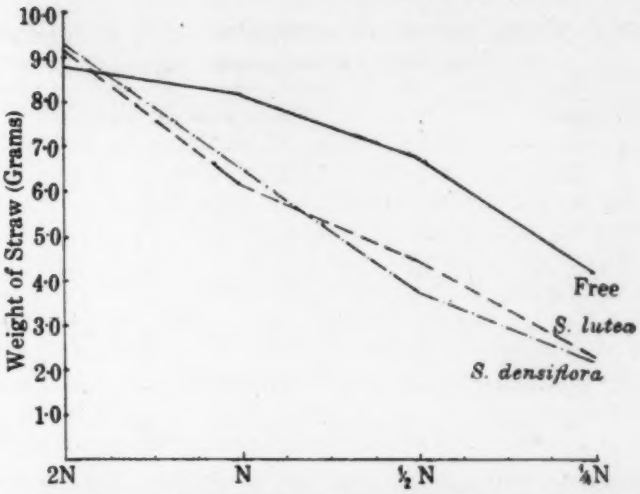

FIG. 4. The effect of four different nutrient concentrations on the height of Straw (in grams) of host plants attacked by *Striga lutea* and *S. densiflora* and uninfected host plants.

analysed separately at each nutrition level and from the results it became apparent that the yield of grain of *Sorghum* plants attacked by *S. lutea* and *S. densiflora* at the ½N and ¼N level of nutrition is significantly less than that of free plants. There is no significant difference in the yield of grain of plants attacked by the two species of the parasite at these two levels. On the other hand, there is no significant difference between the number of grains produced by *Sorghum* plants whether attacked by *S. lutea*, *S. densiflora* or free at the 2N and N nutrition level.

The data for the yields of straw was similarly analysed and it was found that there was no significant difference in the weight of straw produced by the *Sorghum* plants whether free or attacked by *S. lutea* or *S. densiflora* at any of the nutrient concentrations tried.

It will be seen that at the highest concentration of the nutrient solution, *i.e.*, 2N, the osmotic pressures of the host and the parasite are very close to each other; the osmotic pressure of *S. lutea* and *S. densiflora* is just a little higher than that of the host and in some cases it is the same as that of the host. This confirms the views expressed by Harris and Lawrence (1916). The data are graphically represented in Fig. 5.

In order that the conclusions to be drawn from these data may be statistically valid, the figures were analysed in three different ways, *viz.*, (*a*) the osmotic pressure of the hosts at different levels of nutrition and at different treatments, (*b*) the osmotic pressure of *S. lutea* and *S. densiflora*

TABLE I. *Osmotic pressure (in atmospheres) of the host and parasite in different nutrient solutions*

Treatments		Osmotic pressure in atmospheres							
Nutrition	*Striga*	1	2	3	4	5	6	Total	Mean
2N	Free-Host ..	8·7	9·3	8·4	9·0	9·6	8·4	53·4	8·9
2N	*S. lutea* Host ..	9·6	8·4	9·6	9·3	8·7	9·0	54·6	9·1
	Parasite ..	9·9	*	9·9	9·3	*	9·0	38·1	9·5
2N	*S. densiflora* Host ..	9·9	8·4	9·3	8·7	9·6	8·7	54·6	9·1
	Parasite ..	*	8·4	9·3	8·7	9·9	*	36·3	9·1
N	Free-Host ..	6·7	7·8	7·0	6·7	7·5	6·4	42·1	7·0
N	*S. lutea* Host ..	8·1	6·4	7·3	6·7	8·1	6·7	43·3	7·2
	Parasite ..	13·0	10·8	12·4	11·7	12·7	8·7	69·3	11·5
N	*S. densiflora* Host ..	8·7	7·3	5·9	6·7	6·4	7·0	42·0	7·0
	Parasite ..	12·7	10·2	11·1	10·5	11·4	11·7	67·6	11·3
½N	Free-Host ..	4·5	4·7	5·3	5·9	6·9	4·7	31·8	5·3
½N	*S. lutea* Host ..	6·7	4·7	4·7	5·9	6·4	4·7	33·1	5·6
	Parasite ..	15·0	12·7	14·6	8·7	11·4	15·6	78·0	13·0
½N	*S. densiflora* Host ..	3·7	5·0	5·6	4·0	3·7	5·6	27·6	4·6
	Parasite ..	10·2	10·5	11·4	10·8	14·3	11·7	68·9	11·5
¼N	Free-Host ..	4·7	5·3	4·7	4·7	4·7	4·2	22·3	4·7
¼N	*S. lutea* Host ..	5·3	5·0	5·3	4·2	4·0	4·7	28·5	4·7
	Parasite ..	12·7	10·8	15·2	12·7	11·7	11·7	74·8	12·4
¼N	*S. densiflora* Host ..	3·7	5·0	3·7	4·7	4·0	4·7	25·8	4·3
	Parasite ..	12·1	12·7	12·7	12·7	11·1	14·6	75·9	12·6

* No parasite

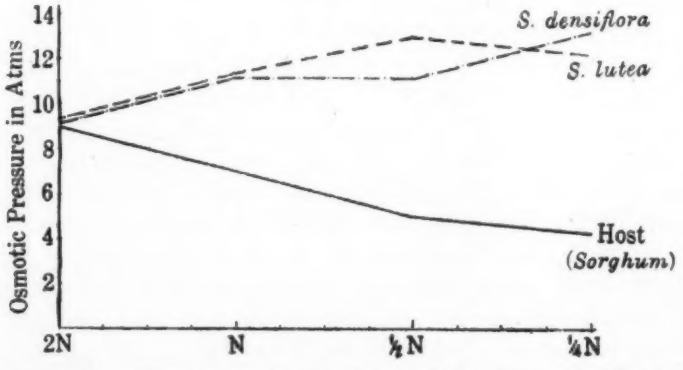

FIG. 5. Osmotic pressure (in atms.) of the leaves of host plants and of *Striga lutea* and *S. densiflora* at four different nutrient concentrations.

at different levels of nutrition, and (*c*) the difference between the osmotic pressure of the parasite and its host at the different nutrient concentrations.

The following conclusions were arrived at from each of these statistical analyses:—

(*a*) The osmotic pressure of *Sorghum* leaves is the highest at the 2N level of nutrition and falls as the concentration of the nutrient solution is lowered, until at the ¼N level it is the least. The difference between the two extreme values is 4·8 (9·1–4·3). The differences between 2N and N and between N and ½N are significant but not between ½N and ¼N concentration of the nutrient solution. The osmotic pressure of the host at each concentration of nutrient medium whether attacked by *S. lutea* or *S. densiflora* or free, does not show differences which are statistically significant. It may, therefore, be concluded that the attack of *Striga* does not influence the osmotic value of the leaves of the host.

(*b*) The osmotic pressure of the parasite is highest at the lowest concentration of the nutrient solution, *i.e.*, ¼N and lowest at the highest concentration, *i.e.*, 2N. The differences between the two extreme values was 3·5. The differences between the ¼N, ½N and N levels are not significant. Comparing the values obtained for the parasite with that of the host, we find that whilst in the host, osmotic pressure rises with the increase in concentration of the nutrient solution, that of the parasite falls. Further it must be pointed out that the range of the values for the host is from 9·6 to 4·3, whereas those for the parasite range from 15·6 to 8·4 atmospheres showing that the osmotic pressure of the parasite is definitely higher than that of the host.

(*c*) The differences between the osmotic pressure of the parasite and the host are largest at ¼N, *i.e.*, 8·3 and 7·7 and slightly, though not statistically less, at ½N, *i.e.*, 6·9 and 7·4; at N level there is a mean difference of 4·3 atmospheres and at the 2N concentration the difference is almost nil. *S. lutea* and *S. densiflora* behave similarly in this respect also.

Discussion

By growing the hosts, *i.e.*, *Sorghum* plants in a series of sand cultures infected with *S. lutea* or *S. densiflora* seed or kept uninfected, and watering them with various concentrations of a nutrient solution, it is seen that the grain yields of *Sorghum* plants attacked by *S. lutea* and *S. densiflora* are significantly smaller than that of free plants at the ½N and ¼N levels of nutrition. The results of the osmotic pressure determinations of the leaves of the hosts and parasites seem to indicate that at these concentrations of the nutrient solution, the osmotic pressure of the parasite is very much

higher than that of the host, there being a difference of 7 to 8 atmospheres between the two. It is quite likely, that at the ¼N and ½N level, the parasite is able to deprive the host of large quantities of water and in consequence the yield of the host is lowered. At the N concentration there is a difference of 4·3 atmospheres between the osmotic pressure of the parasite and that of its host. When the grain yield of free and attacked plants is compared at this level, it is found that the differences in yield are not significant. It would, therefore, appear that at this concentration the quantity of food material removed by the parasite is not large enough to lower the yield of the host. At the 2N level, the osmotic pressure of the hosts and parasites is practically the same. At this concentration, it seems, the parasite leads a very precarious existence. This is the only concentration in which all the *Sorghum* plants are not attacked by the parasite. Even if the *Striga* plants do appear they die very quickly, almost invariably without flowering. It must also be noted that the osmotic pressure of the parasites is the lowest at this concentration. This is contrary to the findings of Parija and Saran (1932) who reported that the sap of the parasite rose in strength as the osmotic pressure of the host was increased. According to the data presented here, the osmotic pressure of the parasite decreased from 12·6 to 11·3 as the sap of the host rose from 4·3 to 7·0. As the differences for the parasite were not significant, it may be concluded that the osmotic pressure of the parasite did not change—certainly did not rise—as that of its host rose. In fact, at the 2N level when the osmotic pressure of the host reached its highest value, that of the parasite fell and this fall is statistically significant. It may, therefore, be concluded that both *S. lutea* and *S. densiflora* have an inherent osmotic value which is much higher than the host when the latter is grown at the ¼N, ½N and N levels of nutrition. But at the 2N level, this value falls to very near that of the host, which is at its peak.

SUMMARY

While studying the influence of nutrition of the host on the susceptibility of *Andropogon sorghum* to attack by the phanerogamic root-parasites *Striga lutea* and *S. densiflora*, the host along with the parasites were grown in sand cultures watered by four different concentrations of the same nutrient solution for a period of nearly five months, *i.e.*, from sowing to harvest. These studies were utilised for testing the ability of the parasite to adjust its osmotic pressure to that of the host.

From the results obtained it was found that the severity of attack was the highest at the lower concentration and lowest at the higher concentration of the nutrient solution. Similarly the damage done by *Striga* plants as

SORGHUM PLANTS GROWING IN SAND CULTURES WATERED BY NUTRIENT SOLUTIONS

2N N ½N ¼N

3rd Row Free, 2nd Row *S. lutea*, 1st Row *S. densiflora*

EFFECT OF *STRIGA* ATTACK ON SORGHUM PLANTS AT 1/4 N NUTRITION SOLUTION

SORGHUM PLANT
Killed by
S. DENSIFLORA

SORGHUM PLANT
Killed by
S. LUTEA

determined by the reduction in yield of grain of the *Sorghum* plants was the greatest at the lower concentrations. The differences in yields of straw were not significant.

The osmotic pressures of the leaves of *Sorghum* plants, attacked by *S. lutea* and *S. densiflora* and free *Sorghum* plants were determined by the plasmolytic method. The osmotic values of the leaves of the parasites were measured at the same time. It was found that the osmotic pressure of the parasite was always much greater than its host at the lower concentrations but at the highest concentration, the host and parasite have practically the same osmotic pressure.

Acknowledgement

I take this opportunity to express my gratitude to Prof. I. S. S. Kumar, M.Sc., A.R.C.S. (Lond.), F.A.Sc., Economic Botanist to Government, State of Bombay, for his valuable guidance throughout the course of the investigations.

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Explanation to Plates

1. *Sorghum* plants growing in sand cultures watered by nutrient solutions. The arrangement in the photograph may be illustrated diagrammatically as follows :—

3rd row Free	0	0	0	0
2nd row *S. lutea*	0	0	0	0
1st row *S. densiflora*	0	0	0	0
	2 N	N	½ N	¼ N

2. Effect of *Striga* attack on *Sorghum* plants at ¼ N nutrition level.
Left — *Sorghum* plants killed by *S. densiflora*.
Right — *Sorghum* plants killed by *S. lutea*.

INTERACTION OF VITAMIN C AND UREASE

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For the first time, Leuthardt and Koller (1934) reported that urease was rendered inactive by the combination of Vitamin C and a Cu-salt, whereas the same concentration of these substances, separately was without effect. Later Bersin and Koster (1935 *a* and 1935 *b*) working with soyabean urease, showed that Vitamin C had no effect on urease, while it inactivated the enzyme when combined with ferrous iron or copper. Contradictory to the above findings, Elson (1943) found that Vitamin C at low concentrations inhibited urease activity and that this inhibition was prevented by the addition of cysteine. Quastel (1943) suggested that the inhibition of the urease activity by Vitamin C was due to the oxidised form of the Vitamin, namely, the diketone (dehydroascorbic) and not to Vitamin C itself and that the inhibition was due to a reaction between dehydroascorbic acid and an –SH group of the enzyme. The disappearance of the inhibition on the addition of cysteine was attributed to the reduction of dehydroascorbic acid to the inert Vitamin C.

In view of these contradictory observations, it seemed desirable to reinvestigate the nature of the inhibition of urease activity by Vitamin C. In an earlier note Giri and Seshagiri Rao (1944), reported that dehydroascorbic acid did not inhibit urease. As there was no inhibition of the enzyme by ascorbic acid in presence of substances (8-hydroxyquinoline and diethyl-dithiocarbamate) which inhibit the oxidation of Vitamin C by Cu^{++} the authors suggested that the inhibition might be due to the formation of intermediary products like Cu_2O. Subsequently Mapson (1946) also observed that neither ascorbic acid in the absence of Cu^{++}, nor dehydroascorbic acid inhibit the enzyme, and showed that ascorbic acid acts as an inhibitor only in so far as it reduces Cu^{++} to Cu^{+}, thereby confirming fully the findings of Giri and Seshagiri Rao (1944).

The present paper deals with a more detailed investigation of the influence of Vitamin C, dehydroascorbic acid and substances which inhibit the oxidation of Vitamin C on urease activity, with a view to examine the mechanism of the interaction of Vitamin C with urease.

Experimental

Preparation of the enzyme

Urease prepared by two different methods was used in all the experiments reported in the present investigation.

(*a*) *Crude urease.*—3 Grammes of fine Jack-bean powder was extracted with 50 c.c. of distilled water by keeping it overnight in the refrigerator with a little toluene at the top. The next day it was filtered and the clear filtrate was used as source of the enzyme.

(*b*) *Pure urease.*—Since the Jack-bean powder contained low amounts of urease, Sumner's modified method (1928) for the preparation of pure urease in Jack-bean meal of low urease content, was adopted.

The Jack-bean powder was defatted by soxhletting with peroxide-free ether. 100 Gm. of the defatted material was mixed well with 500 c.c. of 31·6% acetone for 5 minutes at 20° C. and filtered by gravity in the refrigerator. To the acetone extract, 10 c.c. of 0·2 N acetic acid were added quickly while stirring and the final extract was kept overnight in the refrigerator. The next day, the precipitated urease was centrifuged off and was washed with 15 c.c. of 32% acetone containing a little phosphate at pH 6·0. The final precipitate was taken up in 15 c.c. of twice-glass distilled water and the solution was then centrifuged to remove any insoluble matter. The final clear supernatant liquid obtained was used as the pure urease.

Determination of the activity of urease

The enzyme was allowed to act upon urea under specified conditions of temperature, pH and substrate concentration and the ammonia formed after 30 minutes hydrolysis was estimated by the usual aeration method of Vanslyke. The urease activity was always expressed in terms of mg. of ammonia formed after 30 minutes hydrolysis of urea by the enzyme.

Twice-glass distilled water, distilled from an all-pyrex distillation still was used throughout this investigation.

All the chemicals used were of the highest grade of purity.

The pH of all the solutions used in testing their influence on the activity of the enzyme, was always adjusted to the pH of the experimental solution.

All the solutions of the substances and of ascorbic acid were always freshly prepared for each experiment.

All the experiments to be reported were conducted using the different preparations of urease—the crude, and the purified. In each case for comparison, the results obtained with the crude and purified enzyme are given in one table.

Influence of Vitamin C on urease

The effect of increasing concentrations of Vitamin C on urease activity was investigated.

The reaction mixtures consisted of 3 c.c. of M/5 acetate buffer (pH 6·0), 0·5 c.c. urease solution, varying amounts of Vitamin C ranging from 1 to 5 mg. and distilled water. The total volume of the reaction mixture in each case was adjusted to 8 c.c. After the mixtures were incubated at 30° C. for 30 minutes, 2 c.c. of 3% urea were added to each and after 30 minutes hydrolysis, the liberated ammonia was estimated. The results are summarised in Table I.

TABLE I

Effect of varying concentrations of Vitamin C on urease

	Vitamin C (mg.)	Mg. of ammonia formed after 30 minutes hydrolysis	
		Crude enzyme	Purified enzyme
Urease alone	..	7·6	9·8
Urease + Vitamin C	1·0	2·4	3·5
do ..	1·5	2·0	2·8
do ..	2·0	1·2	2·0
do ..	4·0	0·8	1·0
do ..	5·0	0·7	1·0

The results indicate that there is gradual inactivation of urease with increasing concentrations of Vitamin C.

Influence of various stabilizers of Vitamin C on the inactivation of urease by Vitamin C

The influence of various substances including purines and creatinine, which are known to inhibit the oxidation of Vitamin C (Giri and Krishna Murthy, 1941; Giri and Seshagiri Rao, 1946) was investigated. The reaction mixtures consisted of 3 c.c. M/5 acetate buffer (pH 6·0), 0·5 c.c

of urease solution, 1 mg. of Vitamin C and solution of the substance whose influence on the inactivation of urease was to be determined. The total volume of the reaction mixtures was always made up to 8 c.c. The mixtures were incubated at 30° C. for 30 minutes. 2 c.c. of 3% urea solution were added to each and the liberated ammonia was estimated after hydrolysis for 30 minutes.

The results are given in Table II.

TABLE II

Influence of stabilizers of Vitamin C on the inactivation of urease by Vitamin C

	Substance added	Concentration of the substance	Mg. of ammonia formed after 30 minutes hydrolysis	
			Crude enzyme	Purified enzyme
Urease alone ..	..	..	7·5	9·7
Urease + Vitamin C ..	..	..	2·4	3·5
do	8-Hydroxy quinoline	$1·72\times10^{-3}$ M	7·5	9·6
do	Sod. diethyl-dithio-carbamate	$1·46\times10^{-3}$ M	7·0	8·9
do	Pot. cyanide	$3·84\times10^{-3}$ M	7·5	9·7
do	Oxalic acid	$4·95\times10^{-3}$ M	2·5	3·5
do	Uric acid	$3·71\times10^{-3}$ M	2·4	3·5
do	Guanine	$4·13\times10^{-3}$ M	2·4	3·5
do	Theophylline	$3·47\times10^{-3}$ M	2·4	3·5
do	Xanthine	$4·1\times10^{-3}$ M	2·4	3·5
do	Creatinine	$5·52\times10^{-3}$ M	4·0	3·8

The results show that the inhibition of urease by Vitamin C can be prevented by substances other than cysteine (8-hydroxyquinoline, sodium diethyl-dithio-carbamate and potassium cyanide) which are known to inhibit the oxidation of Vitamin C by Cu^{++}. This points to the possibility that the inhibition of urease activity by Vitamin C is due to the oxidation of the Vitamin by traces of Cu that may be present in the reaction mixture. It is, therefore, suggested that the function of Cysteine in protecting the urease from inactivation by Vitamin C (Elson, 1943) also consists in its combination with traces of Cu in the reaction mixture, thereby preventing the oxidation of the Vitamin. Among the purines tested, only creatinine protected the crude urease from inactivation to a small extent while it has no effect on the inactivation of the purified enzyme. But all the other purines and oxalic acid are without any effect on the inactivation of the crude as well as the purified urease by Vitamin C.

The effect of dehydroascorbic acid on urease was next investigated to obtain further evidence regarding the mechanism of inactivation of urease by Vitamin C.

Preparation of dehydroascorbic acid

Dehydroascorbic acid was prepared by two different methods:

(1) by oxidation with norite (Fox and Levy, 1936)

(2) by oxidation with the enzyme 'Ascorbic acid oxidase'.

The results are reported in Table III.

TABLE III

Influence of Vitamin C and dehydroascorbic acid on urease activity

Experimental mixture	Mg. of ammonia formed after hydrolysis for 30 minutes	
	Crude enzyme	Purified enzyme
Urease alone ..	7·5	9·6
Urease + Vitamin C ..	2·3	3·4
Urease + dehydroascorbic acid (Prepared by Norite method)	7·0	9·0
Urease + dehydroascorbic acid (Prepared by oxidation with ascorbic acid oxidase)	6·9	9·0

The reaction mixtures consisted of 3 c.c. M/5 acetate buffer (pH 6·0), 0·5 c.c. urease solution, 1 mg. of Vitamin C or dehydroascorbic acid corresponding to 1 mg. of the vitamin, the total volume of the mixture being 8·0 c.c. At the end of 30 minutes incubation at 30° C., 2 c.c. of 3% urea solution were added to the mixtures and the ammonia formed after 30 minutes hydrolysis at 30° C. was estimated.

Contrary to the suggestion of Quastel (1943), the above results clearly indicate that the inhibiting action of Vitamin C is not due to dehydroascorbic acid as the degree of inhibition exerted by dehydroascorbic acid is negligible compared with that exerted by Vitamin C at equal concentration. These results support the view already expressed by the authors in the case of β-amylase (Seshagiri Rao and Giri, 1942), that the inhibition of the enzyme by Vitamin C is related to the oxidation of the vitamin and is not due to dehydroascorbic acid.

Effect of copper on urease activity

The influence of varying concentrations of copper on urease activity was investigated.

The reaction mixtures contained 3 c.c. M/5 acetate buffer (pH 6·0), 0·5 c.c. urease solution, varying amounts of $CuSO_4.5H_2O$ solution containing from 0·2-10·0 γ Cu^{++}, and distilled water to adjust the total volume to 8 c.c. After incubation for 30 minutes at 30° C., 2 c.c. of 3% urea were added to the mixture and the ammonia formed after 30 minutes hydrolysis was estimated. The results are summarized in Table IV.

TABLE IV

Influence of varying concentration of Cu^{++} on urease activity

Experimental mixture	Mg. of ammonia formed after 30 minutes of hydrolysis	
	Crude enzyme	Purified enzyme
Urease alone	7·4	9·4
Urease + Cu (0·2 γ) ..	7·2	9·3
do (0·5 γ) ..	6·8	8·6
do (1·0 γ) ..	4·5	6·0
do (2·0 γ) ..	3·8	5·4
do (5·0 γ) ..	3·0	4·0
do (10·0 γ) ..	1·8	2·4

The results show that copper at higher concentrations is toxic to urease. At a concentration of 0·2 γ Cu^{++} there is practically no inhibition. This concentration of Cu^{++} was used in the subsequent experiments to find out the effect of Vitamin C–Cu complex on urease activity.

Effect of Vitamin C–Cu complex on urease

Such concentrations of Vitamin C and Cu^{++} which exert little inhibition on urease when tested individually, were used in the following experiment.

The reaction mixtures containing 3 c.c. M/5 acetate buffer (pH 6·0), 0·5 c.c. urease solution, $CuSO_4.5H_2O$ solution (containing 0·2 γ Cu^{++}) and 0·5 mg. of Vitamin C, in a total volume of 8 c.c., were incubated at 30° C. for 30 minutes and at the end of the period, 2 c.c. of 3% urea solution were added, and the ammonia formed during 30 minutes hydrolysis was

estimated. Proper controls were always run. The results are summarised in Table V.

TABLE V

Influence of Vitamin C–Cu complex on the activity of urease

Experimental mixture	Mg. of ammonia formed after 30 minutes hydrolysis	
	Crude enzyme	Purified enzyme
Urease alone ..	7·4	9·5
Urease + Cu^{++} (0·2 γ) ..	7·2	9·2
Urease + Vitamin C (0·5 mg.) ..	5·3	6·2
Urease + Vitamin C + Cu^{++} ..	1·4	2·0

The results show that the urease is inhibited to a very marked extent by Vitamin C–Cu complex as compared to that exerted by Cu^{++} or Vitamin C alone.

Study of the correlation between Vitamin C oxidation and urease inactivation

To prevent any further oxidation of the Vitamin in the digestion mixture, a stabiliser, 8 hydroxy-quinoline, was added to all the digestion mixtures.

The following experimental mixtures were set up containing:

(1) 10 c.c. M/5 acetate buffer (pH 6·0) + 5 c.c. urease solution + 5 c.c. water

(2) do. + 5 c.c. urease solution + 3 c.c. Vitamin C solution (10 mg.) + 2 c.c. water

(3) do. + 5 c.c. urease solution + 3 c.c. Vitamin C (10 mg.) + 2 c.c. 8-hydroxy-quinoline (20 mg.)

The mixtures were incubated at 30° C. and at stated intervals of time 2 c.c. aliquots were removed and added to the digestion mixtures for the determination of the urease activity. Simultaneously, the Vitamin C content in those aliquots was also estimated by titration with the dye.

The digestion mixture always consisted of 3 c.c., M/5 acetate buffer (pH 6·0), 2 c.c. 3% urea solution 2 c.c., 8-hydroxy-quinoline (2 mg.) and 1 c.c. distilled water.

The results are summarised in Table VI (A) and VI (B).

TABLE VI (A)

Relation between the oxidation of Vitamin C and inactivation of urease treated with the vitamin in presence and absence of 8-hydroxy-quinoline

Time of incubation in minutes	Crude urease					
	Enzyme alone		Enzyme + Vitamin C		Enzyme + Vitamin C + 8-hydroxy-quinoline	
	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot
0	7·4	..	7·4	1·0	7·4	1·0
15	7·4	..	3·0	0·96	7·4	1·0
30	7·4	..	2·3	0·86	7·3	1·0
60	7·3	..	2·0	0·75	7·3	1·0

TABLE VI (B)

Time of incubation in minutes	Purified urease					
	Enzyme alone		Enzyme + Vitamin C		Enzyme + Vitamin C + 8-hydroxy-quinoline	
	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot	Activity in mg. ammonia formed after 30 minutes hydrolysis	Mg. of Vitamin C in the 2 c.c. aliquot
0	9·4	..	9·4	1·0	9·4	1·0
15	9·4	..	4·5	0·95	9·4	1·0
30	9·4	..	3·5	0·8	9·4	1·0
60	9·4	..	2·0	0·7	9·3	1·0

The above results clearly indicate that the inhibition of the activity of urease by Vitamin C is closely related to the oxidation of the vitamin.

The influence of various stabilizers of Vitamin C oxidation on the inactivation of urease by Vitamin C–Cu complex was next investigated and the results obtained are reported in Table VII.

TABLE VII

Influence of stabilisers on the inactivation of urease by Vitamin C–Cu complex

	Substance added	Concentration of the substance 10^{-3} M	Mg. of ammonia formed after 30 minutes hydrolysis	
			Crude enzyme	Purified enzyme
Urease alone ..	..	..	7·3	9·4
Ureasee + Vitamin C + Cu^{++}	..	..	1·4	2·0
do ..	8·0 H-quinoline	1·72	7·2	9·2
do ..	Sod. diethyl-dithio-carbamate	1·46	7·0	9·0
do ..	Pot. cyanide	3·84	7·3	9·3
do ..	Oxalic acid	4·95	2·0	2·2
do ..	Uric acid	3·71	1·8	2·0
do ..	Xanthine	4·1	2·0	2·5
do ..	Theophylline	3·47	1·8	2·1
do ..	Creatinine	5·52	2·5	2·8

The reaction mixtures consisted of 3 c.c. M/5 acetate buffer (pH 6·0) 0·5 c.c. urease solution, $CuSO_4 \cdot 5H_2O$ solution containing 0·2 γ Cu^{++}, Vitamin C solution (containing 0·5 mg.) and water or solution of the substance whose influence on the inactivation of urease was to be determined. The total volume of the reaction mixture was always adjusted to 8 c.c. To the mixtures, after incubation for 30 minutes at 30° C., 2 c.c. of 3% urea were added and the ammonia formed after a subsequent 30 minutes hydrolysis was determined.

The results show that the inactivation of urease by Vitamin C–Cu complex is annulled by 8-hydroxy-quinoline, sodium diethyl-dithio-carbamate and potassium cyanide, while oxalic acid and the purines characteristically do not exert any protection against the inactivation of the enzyme by Vitamin C–Cu complex.

DISCUSSION

The results reported here show that the inhibiting action of Vitamin C on urease (Elson, 1943) is not due to dehydroascorbic acid, as the degree of inhibition exerted by dehydroascarbic acid is negligible compared with

that exerted by Vitamin C at equal concentration. This observation is not in conformity with the suggestion put forward by Quastel (1943), namely, that the inhibition of urease by Vitamin C is due to the formation of dehydroascorbic acid. Furthermore, the fact that the inhibition of urease by Vitamin C can be prevented by substances other than cysteine, which are known to inhibit the oxidation of Vitamin C by forming complexes with Cu^{+} tends to show that the inhibition is due to the oxidation of the vitamin by traces of copper present in the reaction mixture. The authors believe that the reported protection by cysteine of urease against inactivation by Vitamin C (Elson, 1943) also consists in its combination with traces of copper in the reaction mixture, thereby preventing the oxidation of the vitamin.

The facts reported in the foregoing clearly indicate a regular correlation between the inactivation of the urease on the one hand and the oxidation of Vitamin C on the other. Urease is found to be inactivated to a greater extent by Vitamin C–Cu complex than by Vitamin C alone and that the inhibition is abolished by a variety of compounds (Table VII) which are known to protect the vitamin against oxidation by Cu^{++}. The inactivation of urease by Vitamin C undergoing oxidation catalysed by traces of copper may be attributed to the intermediate products such as Cu_2O formed during the oxidation (Giri and Seshagiri Rao, 1944; Mapson, 1946).

The characteristic absence of any protection against the inactivation of urease by Vitamin C and Vitamin C–Cu complex in the presence of purines may be due to any of the following causes: (1) The purines studied may have a preferential affinity to the unspecific proteins associated with the urease molecule and thus are not made available for blocking traces of Cu^{+} responsible for oxidizing the vitamin or (2) the traces of copper present may be in combination with a protein associated as an impurity with the urease, and the purines may not exert any protection against oxidation of Vitamin C by such a Cu-protein in a similar manner as that observed by Giri and Seshagiri Rao (1946) in the case of their effect on the oxidation of Vitamin C by ascorbic acid oxidase.

Further extensive investigations are necessary before anything definite could be said regarding the nature of the differences observed in the protective action exerted by the various substances investigated, against the inhibitory action of Vitamin C on urease.

Summary

Vitamin C and Vitamin C–Cu complex inhibit urease activity, the inhibition being very marked in the latter case.

The inhibition of urease activity of Vitamin C is not due to dehydro-ascorbic acid as the degree of inhibition produced by it is negligible compared to that produced by Vitamin C at equal concentration.

A variety of compounds other than cysteine, like 8-hydroxy-quinoline, sodium-diethyl-dithio-carbamate and potassium cyanide, which are known to protect Vitamin C against oxidation catalysed by Cu^{++}, are shown to protect the urease from inactivation by Vitamin C and Vitamin C–Cu, showing that the inactivation of urease by Vitamin C is related to the oxidation of the vitamin.

A well marked correlation between the inactivation of urease on the one hand and the oxidation of Vitamin C on the other has been established.

It is suggested that the inactivation of urease by Vitamin C catalyzed by Cu^{++} may be due to any intermediate products like Cu_2O formed during the oxidation.

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